

DATA EVALUATION RECORD

MCPA 2-EHE

Study Type: OPPTS 870.6300 [§83-6], Developmental Neurotoxicity Study in Rats

Work Assignment No. 6-1-254 (MRID 48060301)

Prepared for

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DATA EVALUATION RECORD

STUDY TYPE: Developmental Neurotoxicity Study – Rat; OPPTS 870.6300
(§83-6); OECD 426 (draft)

PC CODE: 030564
TXR #: 0055357

DP BARCODE: D377234

TEST MATERIAL (PURITY): MCPA 2-EHE (97.5% a.i.)

SYNONYMS: 4-Chloro-2-methyl-phenoxy acetic acid 2-ethylhexylester

CITATION: Schneider, S., W. Kaufmann, V. Strauss *et al.* (2010) MCPA 2-EHE:
Developmental neurotoxicity study in Wistar rats administration to the dams and
pups via the diet. Experimental Toxicology and Ecology, BASF SE,
Ludwigshafen, Germany. Laboratory Project ID: 67R0351/07096, March 25,
2010. MRID 48060301. Unpublished.

SPONSOR: MCPA Task Force Three, 8325 Old Deer Trail, Raleigh, NC 27615

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (MRID 48060301),
MCPA 2-EHE (97.5% a.i., Batch # 09-08-38) was administered continuously in the diet to 40
mated female Wistar rats per dose at nominal dose levels of 0, 300, 900, or 1800 ppm during
gestation and 0, 200, 600, or 1200 ppm during lactation (equivalent to 28, 83, and 156
mg/kg/day, gestation/lactation) from gestation day (GD) 6 through lactation day (LD) 21.

Dams were allowed to deliver naturally and were killed on LD 21, following weaning of their
respective litters. Ten pups/sex/group were allocated to Subsets 1-6 for neurobehavioral testing
(Subsets 3, 4, 5, and 6), and neuropathological examination (Subsets 1, 2, and 3). Additionally, 5
pups/sex/group were allocated to Subsets 7-9 for blood and carcass sampling/analysis of MCPA-
related analytes on PND 4, 11, and 21, respectively.

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Treatment-related effects in the **dams** included the following. Body weights were decreased by 4% on GD 20 at 1800 ppm. Also at this dose, body weight gains were decreased by 12-22% during GD 6-13 and 13-20 following initiation of treatment and by 11% during the overall gestation period (GD 0-20). During lactation, body weights were decreased by 5% on LD 7, and body weight gains were decreased by 31% during LD 1-7 at 1200 ppm. Additionally at this dose, food consumption was decreased by 9% each during GD 6-13 and 13-20 and by 10-21% during all lactation intervals. However, no significant decreases in food consumption were observed for the overall gestation (GD 0-20) or lactation (LD 1-21) periods.

In the **pups**, at 1800 ppm, body weights (17-25%) and body weight gains (15-32%) were decreased in males and females combined at all pre-weaning intervals. During post-weaning, body weights generally remained decreased at most intervals throughout the study in both sexes in Subsets 3-6 in the 1800 ppm group. Body weight gains were often decreased during PND Week 1, but showed signs of recovery throughout the remainder of the study in the animals maintained for 5-6 weeks post-weaning (Subsets 3, 4, and 6). Terminal body weights were decreased by 26-29% in both sexes at this dose on PND 22 and by 12% in the males on PND 60 (+/-2).

The numbers of pups dead and cannibalized were increased at this dose. Most of these pups died within 4 days after parturition, but losses continued until the end of the second week of lactation. Even though the complete litter loss of dam #124 was due to lack of nursing, the overall increase in pup mortality at this dose was considered to be related to treatment.

The results provide clear evidence that the pups were exposed to the test material and/or its metabolites. The test material (MCPA 2-EHE) was not detectable in the plasma of the dams or pups. However, internal exposure was demonstrated by the considerable concentrations of MCPA and minor amounts of 2-HMCPA in the dams and their pups. The plasma concentrations of the metabolites were dose dependent and relatively constant during the last days of gestation and (except in the low dose) throughout lactation in the dams. The plasma concentrations of the metabolites were dose dependent and increased during the course of the lactation period. There were no marked differences between the male and female pups.

No evidence of developmental neurotoxicity was observed at any dose.

The maternal LOAEL is 1800 ppm (equivalent to 156 mg/kg/day); based on decreases in body weight, body weight gain, and food consumption. The maternal NOAEL is 900 ppm (equivalent to 83 mg/kg/day).

The offspring LOAEL is 1800 ppm (equivalent to 156 mg/kg/day), based on decreases in body weight and body weight gain and an increase in pup mortality. The offspring NOAEL is 900 ppm (equivalent to 83 mg/kg/day).

This study is classified **Acceptable/Non-Guideline** and may be used for regulatory purposes, however, it does not satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300; OECD 426) at this time pending a comprehensive review of all available positive control data.

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COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** MCPA 2-EHE

Description: Yellowish liquid

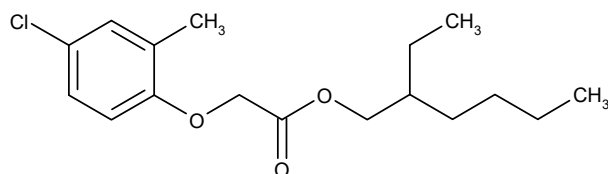
Batch #: 09-08-38

Purity: 97.5% a.i.

Stability: The test material was shown to be stable in the diet for up to 35 days at room temperature.

CAS # of TGAI: 29450-45-1

Structure:



2. **Vehicle:** Diet

3. **Test animals (P)**

Species: Rat

Strain: Wistar (CrI:WI(HAN))

Age at study initiation: Approximately 10-12 weeks

Group mean body weights on GD 0: 165.0-167.3 g females only

Source: Charles River Laboratories, Research Models and Services (Germany)

Housing: Individually in Makrolon type M III cages.

Diet: Kliba maintenance diet mouse/rat "GLP" (Provimi Kliba SA, Kaiseraugst, Switzerland), *ad libitum*, except during behavioral testing.

Water: Tap water, *ad libitum*, except during neurobehavioral testing

Environmental conditions Temperature: 20-24°C

Humidity: 30-70%

Air changes: 10 or 15/hr

Photoperiod: 12 hrs light/12 hrs dark

Acclimation period: 6 days

B. PROCEDURES AND STUDY DESIGN

1. **In-life dates:** Start: 05/11/08 End: 08/05/08

2. **Study schedule:** The maternal animals were time-mated at the breeder and assigned to study. The test substance was administered to the dams from gestation day (GD) 6 through lactation day (LD) 21. After parturition, only the litters containing ≥ 8 pups and whose littering date was over a period of 5 consecutive days (May 26-30, 2008) were kept for further examinations. All other litters and all P females without litters were sacrificed, necropsied, and were discarded without further examinations. On PND 4, the litters were standardized to 8 pups/litter (4 pups/sex where possible) to reduce the variability. Subsequently, one male or one female pup from each litter was randomly allocated to Subsets 1-6 (10 pups/sex/group) and 7-9 (5 pups/sex/group). Litters not selected were kept as reserve animals until PND 21, and were then sacrificed along with all remaining dams. Selected F1 pups remained on the study up until PND 62.

3. **Mating procedure:** The animals were paired by the breeder ('time mated'). Successful mating was verified by the presence of a copulatory plug or sperm in a vaginal smear. The animals were supplied on the same day that successful breeding was determined (GD 0).
4. **Animal assignment:** Time-mated females were randomly assigned to test groups as shown in Table 1. Offspring were assigned to testing subgroups at the time of litter standardization on PND 4. Dams were assigned to functional observation testing as shown.

TABLE 1. Study design ^a					
Experimental Parameter	Subset	Dose (ppm) ^b			
		0/0	300	900	1800
Maternal Animals					
No. of dams assigned	NA	40	40	40	40
FOB (GD 7 & 14, LD 7 & 14)	NA	10	10	10	10
Offspring (F ₁)					
Motor activity (PND 13, 17, 22, & 60)	4	10/sex	10/sex	10/sex	10/sex
FOB (PND 4, 11, 21, 35, 45, & 60)	4	10/sex	10/sex	10/sex	10/sex
Auditory startle habituation (PND 24 and 60)	3	10/sex	10/sex	10/sex	10/sex
Water maze (PND 23 and 30)	5	10/sex	10/sex	10/sex	10/sex
(PND 60 and 67)	6	10/sex	10/sex	10/sex	10/sex
Perfusion, brain weights, neuropathology, and morphometric analysis (PND 11)					
(PND 22)	1	10/sex	10/sex	10/sex	10/sex
(PND 62)	2	10/sex	10/sex	10/sex	10/sex
	3	10/sex	10/sex	10/sex	10/sex
Blood and carcass sampling (PND 4)	7	5/sex	5/sex	5/sex	5/sex
(PND 11)	8	5/sex	5/sex	5/sex	5/sex
(PND 21)	9	5/sex	5/sex	5/sex	5/sex

a Data obtained from page 33-36 of the study report.

b Dose during gestation/lactation

NA Not applicable

5. **Dose selection rationale:** Dose levels were as requested by the Sponsor, and were chosen based on the results of several studies (study numbers not provided) including metabolism studies, 1- and 2-generation studies, a subchronic neurotoxicity study, and a range-finding plasma level study. A higher inclusion rate is specified for the gestation period with a compensatory reduction for the lactation period to ensure an approximately constant parental dose throughout the study. Based on the results of all these studies, doses of 0, 300, 900, and 1800 ppm during gestation and 0, 200, 600, and 1200 ppm during lactation were chosen for the current study.

6. **Dosage preparation and analysis:** Formulations were prepared twice during the study (once each for the gestation and lactation periods) by mixing the appropriate amount of test substance with a small amount of diet to form a premix. This premix was then further diluted with additional diet to achieve the appropriate concentrations. Prior to initiation of the study, stability for up to 35 days at room temperature was determined. Homogeneity and actual concentration at each dose was tested during the gestation and lactation periods.

Results

Stability analysis (% of Day 0): 93.8%

Homogeneity analysis (% CV): 0.4-3.6%

Concentration analysis (% of nominal): 90.8-100.7%

The analytical data indicated that the mixing procedure was adequate and the variation between nominal and actual dosage to the animals was acceptable.

C. OBSERVATIONS

1. In-life observations

- a. **Maternal animals:** The dams were checked for mortality twice daily (once daily on weekends and holidays), and once daily for clinical signs of toxicity. Additionally, parturition and lactation behavior of the dams were checked in the mornings and an additional check for parturition behavior was performed in the afternoon. Body weights and food consumption were measured on GD 0, 6, 13, and 20, and LD 1, 7, 14, and 21.

Ten dams/dose were subjected to a modified functional observation battery (FOB) outside of the home cage on GD 7 and 14, and on LD 7 and 14. It was not reported if the technicians were blind as to the dose group; however, it was stated that tattoo numbers were allocated in a randomized sequence to guarantee the impartiality of the investigator in the open field observation. Details of the scoring for the modified FOB were presented on pages 872-879 of the study report. Observations included, but were not limited to, the following parameters.

FUNCTIONAL OBSERVATIONS	
X	Signs of autonomic function, including: 1) Lacrimation and salivation 2) Nasal discharge 3) Urination and defecation 4) Respiration 5) Palpebral closure 6) Pupil size
X	Description, incidence, and severity of any convulsions, tremors, or abnormal movements.
X	Description and incidence of posture and gait abnormalities
X	Description and incidence of any unusual or abnormal behaviors, activity/arousal levels, altered fur or skin appearance.

b. Offspring

1. **Litter observations:** On the day of birth, the status (sex, liveborn or stillborn) and number of all delivered pups were determined as soon as possible, and all pups were examined macroscopically for any changes. Pups were evaluated for mortality and morbidity twice daily (once daily on weekends and holidays). Clinical observations were recorded daily. Body weights were recorded on PNDs 1, 4 (before standardization), 7, 14, and 21, and then weekly thereafter until sacrifice. Food consumption was not reported.

On PND 4, the litters were standardized by randomly selecting 8 pups/litter; excess pups were sacrificed and discarded. Of the remaining pups, one male or one female pup from each litter was randomly allocated to Subsets 1-6 (10 pups/sex/group) and 7-9 (5 pups/sex/group) for further evaluation.

2. **Developmental landmarks:** Beginning on PND 38, male offspring (except Subset 5) were examined daily for preputial separation. Beginning on PND 27, female offspring (except Subset 5) were examined daily for vaginal patency. The exact days of preputial separation or vaginal patency were recorded, and the body weight of the respective animals on these days was recorded.
3. **Post-weaning observations:** After weaning on PND 21, offspring were examined for mortality and morbidity twice daily (once daily on weekends and holidays). Clinical observations and body weights were recorded weekly until sacrifice. Additionally, body weights were determined on the day of preputial separation or vaginal patency.
4. **Neurobehavioral evaluations:** Observations and the schedule for those observations are summarized as follows from the report.
 - i. **Functional observational battery (FOB):** On PNDs 4, 11, 21, 35, 45, and 60, the Subset 4 animals (10 pups/sex/dose) were subjected to a modified FOB in the open-field, as appropriate for the developmental stage being observed. The same parameters assessed in the maternal FOB were examined in the offspring. It was not reported if the technicians were blind as to the dose group; however, it was stated that tattoo numbers were allocated in a randomized sequence to guarantee the impartiality of the investigator in the open field observation.
 - ii. **Motor activity testing:** Motor activity measurements were performed on animals in Subset 4 (10 pups/sex/dose) on PND 13, 17, 22, and 60 using the Tru Scan Photobeam Linc (Coulbourn Instruments, L.L.C., Allentown, PA) in a darkened room. It was not stated if the technicians were blind as to the dose group. Data were collected in five-minute intervals over the course of 60 minutes. Total movement distance (cm) and number of rears were evaluated.
 - iii. **Auditory startle habituation:** Auditory startle response and habituation of responses with repeated presentation of stimuli were evaluated for animals in Subset 3 (10 pups/sex/dose) on PNDs 24 and 60. The rats were tested using the SR-LAB; Startle Response System (San Diego Instruments, San Diego, CA). The rats were initially given an acclimation period of five minutes within the chamber with 70 dBA background noise. The rats were then

presented with 50 msec, 120 dBA bursts of noise at 5-10 second intervals for 50 trials. It was not reported if any "blank" (baseline) trials were performed. The peak amplitude and latency to the peak of the response were analyzed in 5 blocks of 10 trials each.

- iv. **Learning and memory testing:** Learning and memory testing was performed on animals in Subsets 5 and 6 (10 pups/sex/dose each). Water maze testing was performed beginning on PNDs 23 (Subset 5) and 60 (Subset 6) and again seven days later for each group.

The water maze test consisted of 3 parts (learning ability in the first week, and memory and relearning ability seven days later). The learning ability phase consisted of 6 trials at 1 hour intervals for each rat. On each test trial, the rat was placed into the starting position (base of a M-maze stem farthest from the two arms) and required to find the escape ladder in the right arm of the M-maze. If the animal found the correct path right away it was scored as a positive. If the animal went the wrong way (whole body in wrong alley), it was scored as a negative; however it was allowed to remain in the water until it found the correct route or it reached the maximum swimming duration (6 minutes per trial). After 1 week, the memory phase was performed (one trial for each animal) using the same animals and the same (right side) escape route. The relearning phase was performed within 1 hour following the memory phase. The same procedure as was used in the learning phase was followed; however, the escape route was then placed on the left arm of the M-maze. It was stated that the initial trials of the learning and relearning phases were acclimation trials and were not evaluated.

5. **Cholinesterase determination:** Not applicable; MCPA belongs to the class of compounds (phenoxy herbicide) that do not cause cholinesterase inhibition.
6. **Toxicokinetics:** On GD 20 and LD 4, 11, and 21, blood samples were collected from 5 dams/dose via the retroorbital venous plexus under isoflurane anesthesia. On PND 4 (culled pups), 11, and 21, blood samples were collected from 5 pups/sex/dose (Subsets 7-9) via the vena cava cranialis after decapitation under isoflurane anesthesia. The pups used were from the dams used for blood collection above. After plasma preparation, the samples were divided into two parts and frozen at approximately -20°C prior to analysis. The analyses were carried out at the Analytical Chemistry Laboratory of Experimental Toxicology and Ecology of BASF SE (Ludwigshafen, Germany). All blood specimens were investigated for MCPA-related target analytes by HPLC/MS analysis.
7. **Postmortem observations**
- a. **Maternal animals:** Dams that did not deliver a litter were sacrificed, and their uteri were stained for 5 minutes with a 10% ammonium sulfide solution according the methods of Salewski (1964). The uteri were rinsed, and the number of implantation sites was recorded for calculation of post-implantation loss. All other dams were sacrificed on PND 21 (after weaning), necropsied, and discarded without further examination.
- b. **Offspring:** All pups culled on PND 4, sacrificed on PND 21, and those from Subsets 4-9 (after conclusion of their investigations) were killed by cervical dislocation and discarded without further examination.

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The offspring (10 pups/sex/dose in each subset) selected for brain weight and neuropathological evaluation were sacrificed on PND 11 (Subset 1), 22 (Subset 2), or 62 (Subset 3) under Isoflo® anesthesia via perfusion fixation with Soerensen phosphate buffer followed by 4% formaldehyde. In Subset 1, the cranium with the brain (unopened) was separated from the trunk and fixed for further examinations. In Subsets 2 and 3, the animals were subjected to gross necropsy with regard to neuropathology. The cranial vault and spinal cord were opened and the skin from both hindlimbs was removed. These carcasses were stored in 4% neutral buffered formaldehyde, and then were subjected to postmortem examinations as described below.

The brains (with olfactory bulbs) were removed, weighed, and measured. Tissues from the control and 1800 ppm groups were examined microscopically. The thickness of the following brain sections was measured: (i) neocortex [frontal and parietal cortices], (ii) caudate nucleus/putamen, (iii) hippocampus, (iv) corpus callosum, and (v) cerebellum. Measurements were carried out on both the right and left sides of the brain, with the exception of the corpus callosum and the cerebellum.

The following CHECKED (X) central and peripheral nervous system tissues were collected (Subsets 2 and 3):

	CENTRAL NERVOUS SYSTEM		PERIPHERAL NERVOUS SYSTEM
	BRAIN		SCIATIC NERVE
X	Olfactory bulb	X	Proximal
X	Prosencephalon with frontal lobe		Distal
X	Diencephalon with parietal lobe		
X	Mesencephalon with occipital lobe and temporal lobe		OTHER
X	Pons		Sural nerve
X	Cerebellum	X	Tibial nerve (proximal and distal)
X	Medulla oblongata		Peroneal nerve
		X	Cervical dorsal root ganglion
	SPINAL CORD	X	Cervical dorsal root fibers
X	Cervical swelling	X	Cervical ventral root fibers
X	Thoracic swelling		Thoracic dorsal root ganglion
X	Lumbar swelling		Thoracic dorsal root fibers
	OTHER		Thoracic ventral root fibers
X	Gasserian ganglion	X	Lumbar dorsal root ganglion
X	Pituitary gland	X	Lumbar dorsal root fibers
X	Eyes (with optic nerve)	X	Lumbar ventral root fibers
X	Skeletal muscle (gastrocnemius)	X	All gross lesions
X	Olfactory epithelium (nasal cavity, level III)		

D. DATA ANALYSIS

- Statistical analyses:** The data were analyzed using the following statistical methods, and the level of significance was reported at $p \leq 0.05$ and $p \leq 0.01$ for all tests:

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Parameter	Statistical Methods
Food consumption (maternal), body weight and body weight gain (maternal and pups, for pups the litter means were used), duration of gestation, # of pups delivered per litter, time to sexual maturity	Dose groups were compared to controls using 2-sided Dunnett's test for the hypothesis of equal means.
Female fertility index, gestation index, # of females with liveborn pups, # of females with stillborn pups, # of females with all stillborn pups, live birth index, # of stillborn pups, # of dead pups, # of pups cannibalized, # of pups sacrificed moribund, water maze evaluation, sexual maturity data, accidental death/gavage error, water maze evaluation	Each dose group was compared pairwise to the control using one-sided Fisher's Exact test for the hypothesis of equal proportions.
Brain weights, motor activity, startle response	A non-parametric Kruskal-Wallis test was performed followed by a 2-sided Wilcoxon test, as necessary.
Morphometric parameters	Each dose group was compared pairwise to the control using 1-sided Wilcoxon test for the hypothesis of equal medians. (Brain width and length: with Bonferroni-Holm-adjustment)

2. **Indices:** The following indices were calculated by the Sponsor:

$$\text{Fertility index (\%)} = \frac{\# \text{ of females pregnant}}{\# \text{ of females mated}} \times 100$$

$$\text{Gestation index (\%)} = \frac{\# \text{ of females with live pups on day of birth}}{\# \text{ of females pregnant}} \times 100$$

$$\text{Live birth index (\%)} = \frac{\# \text{ of live pups at birth}}{\text{total \# of pups born}} \times 100$$

The reviewers calculated the following indices using the formulas below and included the data in the summary tables.

$$\text{Viability index (\%)} = \frac{\# \text{ live pups on PND 4 (pre cull)}}{\# \text{ live pups on PND 0}} \times 100$$

$$\text{Lactation index (\%)} = \frac{\# \text{ live pups on PND 21 (weaning)}}{\# \text{ live pups on PND 4 (post cull)}} \times 100$$

3. **Positive and historical control data:** Positive control data were not provided with the current study. However, summaries of seven studies (MRIDs 45540501 through 45540507) performed to generate positive control data and validate the procedures and observers of the performing lab to conduct the FOB and to assess motor activity, neurotoxicity and behavioral effects, previously reviewed by the Agency, were obtained by the reviewers. Exposure to **3,3-iminodipropionitrile** (2000 mg/kg, single i.p. dose) induced the following in both sexes: (i) decreased body weight; (ii) FOB effects (eg. ataxia, females only); (iii) decreased fore- and hindlimb grip strength; (iv) corneal opacities; (v) blood lacunae in the iris; (vi) anisocoria; and (vii) hematobulbus. Additionally, the following histopathological effects were noted: (i) axonal atrophy in the distal segments of the tibial, sural, and sciatic nerves;

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(ii) intraocular hemorrhage; (iii) retinal degeneration with atrophy; and (iv) degeneration and atrophy of the optic nerve. **Acrylamide** (40 mg/kg, 11 daily gavage doses in 2 weeks) induced the following in both sexes: (i) abnormal gait (ataxia, splay of toes of the hindlimbs and/or splay of the hind limbs); (ii) decreased fore- and hindlimb grip strength; and (iii) increased hindlimb foot splay. Additionally in the males, body weight and body weight gains were decreased, and decreased activity, reduced tail pinch response, and increased reaction time to hot-plate test were observed. In addition to decreased brain weight in both sexes, the following histopathological effects were noted: (i) selective Purkinje cell necrosis and vacuolation of the molecular layer of the cerebellar cortex; (ii) cytoplasmic remodeling in the lumbar spinal ganglia cells which resembles chromatolysis; (iii) Wallerian-like axonal degeneration of the sciatic, sural, tibial, and plantar nerves; (iv) neurofilament accumulation, decrease in or loss of synaptic vesicles, and swelling of synaptic terminals in the gastrocnemius muscle; and (v) neuronal necrosis in the mesencephalic trigeminal nucleus region of the midbrain in one male. In addition to the effects given above, acrylamide (30 mg/kg, daily gavage doses up to 4 weeks) induced mortality in both sexes. **Trimethyltin chloride** (6, 9, or 12 mg/kg, single i.p. dose) induced ataxia, tremors, convulsions, decreased grip strength, increased foot splay, and increased motor activity. Additionally, the following neuropathological effects were noted: (i) neuronal necrosis of the olfactory bulbs and midbrain; (ii) axonal degeneration of the cervical ganglia and peripheral nerves; (iii) hydrocephalus internus of the frontal and parietal lobes; (iv) Purkinje cell necrosis in the pons with cerebellar cortex, mid-cerebellum, and medulla oblongata; (v) chromatolysis of alpha motor neurons in the cervical and lumbar spinal cord; and (vi) vacuolar degeneration of the lumbar ganglia. Inter-observer reliability was demonstrated using **carbaryl** (10 or 30 mg/kg, single i.p. dose), **nomifensin** (10 mg/kg, gavage on 2 days), and **diazepam** (3 mg/kg, i.p. on 2 days). All observers detected the FOB effects from carbaryl (abnormal body posture, tremors, repetitive chewing, impaired gait, and reduced rearing), the increased motor activity from nomifensin, and the decreased motor activity from diazepam.

II. RESULTS

A. PARENTAL ANIMALS

1. **Mortality, clinical signs, functional observations, and necropsy:** No treatment-related maternal mortalities were observed during the study. No treatment-related clinical signs were observed during gestation or lactation. No adverse effects were observed in any FOB parameter at any time point. No treatment-related gross lesions were observed at necropsy.
2. **Body weight and food consumption:** Selected group mean body weights, body weight gains, and food consumption for pregnant and nursing dams are summarized in Table 2.

At 1800 ppm, body weights were decreased ($p \leq 0.01$) by 4% on GD 20. Body weight gains were also decreased ($p \leq 0.01$) by 12-22% during GD 6-13 and 13-20 following initiation of treatment and by 11% during the overall gestation period (GD 0-20). During lactation, body weights were decreased ($p \leq 0.01$) by 5% on LD 7, and body weight gains were decreased ($p \leq 0.01$) by 31% during LD 1-7 at this dose. Also at this dose, food consumption was decreased ($p \leq 0.01$) by 9% each during GD 6-13 and 13-20 and by 10-21% during all

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lactation intervals. However, no significant decreases were observed for the overall gestation (GD 0-20) or lactation (LD 1-21) periods.

The decreases ($p \leq 0.05$) noted in body weight gain ($\downarrow 8-11\%$) in the 900 ppm dams during GD 6-13 and 6-20 were minor and did not result in corresponding decreases in body weight.

Food consumption was decreased ($p \leq 0.01$) by 10% during LD 1-7 at this dose.

No treatment-related effects on body weights, body weight gains, or food consumption were observed at 300 ppm during gestation or lactation.

TABLE 2. Selected mean (\pmSD) body weights, body weight gains, and food consumption in dams exposed to MCPA 2-EHE in the diet from GD 6 through LD 21. ^a				
Observations	Dose (ppm)			
	0	300	900	1800
Gestation (n=35-40)				
Body weight (g)				
GD 0	166.9 \pm 11.57	165.0 \pm 10.42	165.0 \pm 11.28	167.3 \pm 10.62
GD 6	198.9 \pm 13.06	197.0 \pm 10.55	197.3 \pm 12.78	199.5 \pm 11.36
GD 20	287.2 \pm 19.93	282.1 \pm 17.64	278.5 \pm 18.79	274.3 \pm 18.01** ($\downarrow 4$)
Body weight gain (g)				
GD 0-6	32.0 \pm 4.81	32.0 \pm 3.57	32.3 \pm 4.86	32.1 \pm 4.05
GD 6-13	29.0 \pm 4.93	27.6 \pm 4.44	25.7 \pm 4.00** ($\downarrow 11$)	22.7 \pm 4.40** ($\downarrow 22$)
GD 13-20	59.3 \pm 7.65	57.5 \pm 9.07	55.5 \pm 8.00	52.2 \pm 8.92** ($\downarrow 12$)
GD 6-20	88.3 \pm 10.85	85.1 \pm 11.53	81.2 \pm 10.2* ($\downarrow 8$)	74.9 \pm 11.79** ($\downarrow 15$)
GD 0-20	120.3 \pm 14.44	117.1 \pm 12.90	113.5 \pm 13.73	107.0 \pm 13.53** ($\downarrow 11$)
Food consumption (g)				
GD 0-6	16.2 \pm 1.28	16.4 \pm 1.25	16.5 \pm 1.30	16.6 \pm 1.13
GD 6-13	18.9 \pm 1.77	18.6 \pm 1.38	18.5 \pm 1.50	17.2 \pm 1.36** ($\downarrow 9$)
GD 13-20	21.1 \pm 1.92	21.0 \pm 1.56	20.3 \pm 1.61	19.2 \pm 2.16** ($\downarrow 9$)
GD 0-20	18.7 \pm 2.42	18.7 \pm 2.28	18.4 \pm 1.90	17.7 \pm 1.37
Lactation (n=32-40)				
Body weight (g)				
LD 1	220.4 \pm 16.51	218.1 \pm 12.08	214.9 \pm 14.29	215.3 \pm 15.21
LD 7	242.9 \pm 17.00	241.8 \pm 11.07	236.7 \pm 13.38	231.9 \pm 15.10** ($\downarrow 5$)
LD 21	251.6 \pm 16.30	250.8 \pm 12.84	247.7 \pm 14.97	249.2 \pm 16.31
Body weight gain (g)				
LD 1-7	22.7 \pm 6.88	23.2 \pm 4.44	22.3 \pm 6.05	15.6 \pm 6.17** ($\downarrow 31$)
LD 7-14	16.1 \pm 7.66	15.0 \pm 8.14	16.3 \pm 6.38	19.4 \pm 6.58
LD 14-21	-7.4 \pm 6.69	-6.1 \pm 8.42	-5.4 \pm 6.70	-2.0 \pm 9.84*
LD 1-21	31.4 \pm 8.88	32.1 \pm 9.72	33.3 \pm 8.97	33.0 \pm 11.78
Food consumption (g)				
LD 1-7	35.5 \pm 3.67	34.1 \pm 2.73	32.0 \pm 4.36** ($\downarrow 10$)	28.0 \pm 4.09** ($\downarrow 21$)
LD 7-14	49.0 \pm 3.67	48.0 \pm 3.81	46.4 \pm 5.86	41.3 \pm 4.26** ($\downarrow 16$)
LD 14-21	56.3 \pm 3.98	55.8 \pm 8.26	54.8 \pm 7.95	50.9 \pm 5.19** ($\downarrow 10$)
LD 1-21	46.9 \pm 10.55	46.0 \pm 11.02	44.4 \pm 11.55	40.1 \pm 11.52

^a Data were extracted from pages 109-114 of the study report. Numbers presented parenthetically are percent difference from controls (calculated by reviewers).

* Significantly different from controls at $p \leq 0.05$

** Significantly different from controls at $p \leq 0.01$

3. **Test substance intake:** Based on maternal food consumption and body weight, the mean dose administered to the presumed pregnant Wistar rats during gestation/lactation was

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approximately 28.2/38.1, 83.3/112.4, and 155.6/204.2 mg/kg/day in the 300, 900, and 1800 ppm groups, respectively.

4. **Reproductive performance:** Reproductive parameters were similar to controls in all dose groups (Table 3). Five control and 4 mid-dose females were not pregnant.

TABLE 3. Reproductive performance ^a				
Observation	Dose (ppm)			
	0	300	900	1800
Number mated	40	40	40	40
Fertility index (%)	88	100	90	100
Gestation index (%)	100	100	100	100
Live birth index (%)	100	100	100	98
Gestation length (mean # of days)	21.5	21.6	21.8	21.8

a Data were extracted from page 118 of the study report.

B. OFFSPRING

1. **Viability and clinical signs:** Litter size and viability results from pups during lactation are summarized in Table 4. There was no treatment-related effect on the mean number of pups/litter or sex ratio. There was a small, but statistically significant, increase ($p \leq 0.05$) in the number and percentage of stillborn pups at 1800 ppm. The percentage of stillborn pups at this dose (1.8%) was within historical limits ($\leq 4.5\%$) and likely reflects the increased litter numbers. The numbers of pups dead and cannibalized were increased ($p \leq 0.01$) at 1800 ppm. Most of these pups died within 4 days after parturition, but losses continued until the end of the second week of lactation. Even though the complete litter loss of dam #124 was due to lack of nursing, the overall increase in pup mortality at this dose was considered to be related to treatment. Plasma concentrations of MCPA in pups exceeded that in dams.

No treatment-related clinical signs of toxicity were observed at any dose. Additionally, no preterminal clinical signs were observed in the 1800 ppm pups that died.

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TABLE 4. Litter size and viability ^a				
Observation	Dose (ppm)			
	0	300	900	1800
No. of litters	35	40	36	40
Total number of pups born	343	370	337	396
Number stillborn	0	0	1	7* (1.8)
Number liveborn	343	370	336	389*
Sex Ratio Day 0 (% male)	49.3	53.5	49.7	47.8
Mean (\pm SD) litter size:				
PND 1 (Total # of pups)	9.8 \pm 2.04 (343)	9.3 \pm 2.28 (370)	9.3 \pm 2.01 (335)	9.5 \pm 1.85 (379)
PND 4 ^b (Total # of pups)	9.7 \pm 2.37 (340)	8.8 \pm 3.07 (351)	9.0 \pm 2.54 (325)	8.9 \pm 2.63 (354)
PND 4 ^c (Total # of pups)	7.4 \pm 1.90 (258)	6.8 \pm 2.66 (271)	7.3 \pm 1.64 (263)	7.2 \pm 2.14 (288)
PND 21 (Total # of pups)	6.3 \pm 2.14 (221)	5.6 \pm 2.81 (222)	5.9 \pm 2.41 (212)	6.2 \pm 2.06 (246)
# Deaths Days 1-4 (%)	0 (0.0)	1 (0.3)	2 (0.6)	31 (8.2)
# Deaths Days 5-21 (%)	1 (0.4)	5 (1.8)	1 (0.4)	12 (4.2)
Number cannibalized (%)	1 (0.3)	5 (1.4)	0 (0.0)	32** (8.1)
Viability index (%) ^d	99.1	94.9	97.0	93.4
Lactation index (%) ^d	85.7	81.9	80.6	85.4

a Data were extracted from pages 119-121 of the study report.

b Pre-culling

c Post-culling

d Calculated by reviewers from data within this table

* Significantly different from controls at $p \leq 0.05$

** Significantly different from controls at $p \leq 0.01$

2. **Body weight:** At 1800 ppm, body weights were decreased ($p \leq 0.01$) by 17-25% in males and females combined at all pre-weaning intervals (Table 5a). Body weight gains in males and females combined were decreased ($p \leq 0.01$) by 15-32% compared to controls throughout all pre-weaning intervals at this dose (Table 5b).

At 900 ppm, pup body weights were not significantly different from controls; however, body weight gains were decreased ($p \leq 0.05$) by 6-7% during PND 4-21 and 14-21.

No treatment-related effects on pre-weaning body weight or body weight gain were noted in the pups at 300 ppm.

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TABLE 5a. Mean (\pm SE) pre-weaning F ₁ pup body weights (g) males and females combined. ^a				
Postnatal Day	Dose (ppm)			
	0	300	900	1800
1	6.4 \pm 0.73	6.4 \pm 0.64	6.4 \pm 0.65	5.3 \pm 0.68** (\downarrow 17)
4 ^b	9.7 \pm 1.15	9.9 \pm 1.27	9.9 \pm 1.19	7.6 \pm 1.28** (\downarrow 22)
4 ^c	9.8 \pm 1.12	10.0 \pm 1.24	9.9 \pm 1.19	7.6 \pm 1.28** (\downarrow 22)
7	15.5 \pm 1.55	15.8 \pm 1.41	15.3 \pm 1.71	11.7 \pm 2.15** (\downarrow 25)
14	32.7 \pm 3.06	33.3 \pm 2.56	31.8 \pm 2.76	26.4 \pm 3.72** (\downarrow 19)
21	51.5 \pm 5.01	51.4 \pm 3.68	49.2 \pm 4.11	40.8 \pm 5.24** (\downarrow 21)

a Data were extracted from on pages 122 and 123 of the study report; n=32-40 litters. Numbers presented parenthetically are percent difference from controls (calculated by reviewers).

b Pre-culling

c Post-culling

** Significantly different from controls at $p \leq 0.01$

TABLE 5b. Selected mean (\pm SE) pre-weaning F ₁ pup body weight gains (g) males and females combined. ^a				
Interval (PND)	Dose (ppm)			
	0	300	900	1800
1-4	3.4 \pm 0.63	3.5 \pm 0.75	3.4 \pm 0.73	2.3 \pm 0.73** (\downarrow 32)
4-7	5.8 \pm 0.63	5.9 \pm 0.86	5.5 \pm 0.78	4.1 \pm 0.99** (\downarrow 29)
7-14	17.2 \pm 1.89	17.4 \pm 1.77	16.5 \pm 1.43	14.6 \pm 2.20** (\downarrow 15)
14-21	18.7 \pm 2.23	18.1 \pm 1.72	17.4 \pm 1.75* (\downarrow 7)	14.4 \pm 1.90** (\downarrow 23)
4-21	41.8 \pm 4.24	41.6 \pm 3.35	39.4 \pm 3.39* (\downarrow 6)	33.1 \pm 4.37** (\downarrow 21)

a Data were extracted from pages 124 and 125 of the study report; n=32-38 litters. Numbers presented parenthetically are percent difference from controls (calculated by reviewers).

* Significantly different from controls at $p \leq 0.05$

** Significantly different from controls at $p \leq 0.01$

As the data were presented in the study report broken down by subset, Table 6 below only contains data from Subset 6 as a representative example of the differences noted in post-weaning body weights and body weight gains.

During post-weaning, body weights generally remained decreased compared to controls at most intervals throughout the study in both sexes in Subsets 3-6 with statistical significance ($p \leq 0.05$ - 0.01) occurring at various intervals in the 1800 ppm group. Body weight gains were often decreased during Weeks 0-1, but showed signs of recovery throughout the remainder of the study in the animals maintained for 5-6 weeks post-weaning (Subsets 3, 4, and 6). With the exception of the Subset 4 males, there were no significant differences in body weight gain for the overall (Weeks 0-5/6) post-weaning period. The decreased ($p \leq 0.01$) body weight gain noted in the 900 ppm males in Subset 6 during Weeks 2-3 post-dosing was considered incidental and unrelated to treatment.

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TABLE 6. Selected mean (\pm SD) post-weaning F ₁ pup body weight and body weight gains (g) from Subset 6. ^a				
Post-weaning Week	Dose (ppm)			
	0	300	900	1800
Males				
0	60.9 \pm 6.78	59.2 \pm 5.01	57.0 \pm 4.44	42.6 \pm 7.80** (\downarrow 30)
3	199.0 \pm 14.95	193.9 \pm 17.41	186.5 \pm 7.13	165.4 \pm 13.91** (\downarrow 17)
6	309.5 \pm 19.75	307.4 \pm 28.14	293.9 \pm 15.23	277.5 \pm 21.13** (\downarrow 10)
0-1	42.5 \pm 4.57	42.3 \pm 4.41	41.3 \pm 2.68	34.0 \pm 3.49** (\downarrow 20)
1-2	49.4 \pm 3.27	48.6 \pm 5.88	47.6 \pm 2.99	44.1 \pm 3.61* (\downarrow 11)
2-3	46.1 \pm 3.65	43.8 \pm 4.97	40.6 \pm 2.09** (\downarrow 12)	42.8 \pm 4.18
Overall (0-6) gain	248.6 \pm 17.14	248.1 \pm 25.52	236.8 \pm 12.86	233.6 \pm 17.01
Females				
0	57.4 \pm 5.55	57.2 \pm 6.78	51.4 \pm 6.36	43.4 \pm 5.93** (\downarrow 24)
2	125.8 \pm 4.74	125.8 \pm 10.61	121.4 \pm 8.86	109.5 \pm 10.45** (\downarrow 13)
6	194.6 \pm 6.65	195.9 \pm 16.61	191.6 \pm 16.45	185.9 \pm 14.44
0-1	35.5 \pm 1.88	36.4 \pm 4.85	35.7 \pm 1.61	30.7 \pm 3.97** (\downarrow 14)
1-2	32.8 \pm 3.43	32.2 \pm 4.34	34.3 \pm 3.50	35.4 \pm 4.36
2-3	20.0 \pm 4.47	21.0 \pm 4.76	22.8 \pm 4.46	25.8 \pm 5.04* (\uparrow 29)
Overall (0-6) gain	137.1 \pm 9.47	138.7 \pm 17.14	140.2 \pm 11.46	142.5 \pm 12.46

a Data were presented in pages 136-151 of the study report; n=10/subset. Data presented in this table are for Subset 6 only from pages 148-151. Numbers presented parenthetically are percent difference from controls (calculated by reviewers).

* Significantly different from controls at $p \leq 0.05$

** Significantly different from controls at $p \leq 0.01$

3. Developmental landmarks

- a. **Sexual maturation:** The time to reach preputial separation was marginally delayed (1.1 days) in the 1800 ppm males (42.3 days treated vs. 41.2 controls, Table 7). The time to reach vaginal patency was also marginally delayed (1 day) at this dose (32.5 days treated vs. 31.5 controls). Body weight at criterion was decreased ($p \leq 0.05$) by 10-11% compared to controls in both sexes. As the delays in both sexes were within the range of historical controls (30.0 to 33.8 days; page 888 of the study report), these findings were not considered adverse, and may be secondary to the decreased body weights noted in these groups, rather than directly related to treatment with the test material.

TABLE 7. Sexual maturation (mean \pm SD day of onset) in F ₁ pups. ^a				
Parameter	Dose (ppm)			
	0	300	900	1800
Preputial separation	41.2 \pm 1.6	41.4 \pm 1.3	41.5 \pm 1.8	42.3 \pm 1.0*
Body weight at criterion (g)	172.0 \pm 6.4	175.4 \pm 11.0	170.7 \pm 11.1	154.8 \pm 10.4** (\downarrow 10)
Vaginal patency	31.5 \pm 2.4	31.2 \pm 1.3	31.7 \pm 1.7	32.5 \pm 1.6
Body weight at criterion (g)	94.1 \pm 15.4	93.8 \pm 10.2	93.9 \pm 9.4	84.1 \pm 9.4* (\downarrow 11)

a Data were extracted from pages 126 and 127 of the study report; n=29-30 for days to criterion and 18-22 for body weight at criterion. Numbers presented parenthetically are percent difference from controls (calculated by reviewers).

* Statistically significantly different from controls at $p \leq 0.05$

** Statistically significantly different from controls at $p \leq 0.01$

- b. **Physical landmarks:** Evaluation of physical landmarks (eye opening, pinna unfolding, incisor erupting) was not performed.

4. **Behavioral assessments**

- a. **Functional observational battery:** No treatment-related FOB effects were observed at any dose in any group.
- b. **Motor activity:** Total session motor activity data (distance traveled and number of rears) are presented in Tables 8a and 8b below. At 1800 ppm, total session distance travelled was decreased compared to controls in both sexes on PND 13 and 17, but was not statistically significant (NS) and was within the historical control range. Similarly, the total session number of rears was decreased ($p \leq 0.05$ -0.01 or NS) by 35-68% in both sexes compared to controls at this dose. At PND 60, motor activity was similar to controls at all doses in both sexes.

The interval data were reported on pages 225-256 and are included as an Appendix in this DER. Several statistically significant differences ($p \leq 0.05$) in activity (distance and rears) were noted at various intervals. All test groups showed normal behavioral ontogeny of motor activity as indicated by an increase in activity at PND 17 followed by a decrease in activity at PND 22, with the exception that motor activity at PND 22 was slightly higher than the activity at PND 17 in the high dose females. Additionally, all groups showed the normal pattern of habituation within session at PND 22 and 60.

TABLE 8a. Mean (\pm SD) total session motor activity (distance travelled, cm) in F ₁ pups. ^a				
Interval (PND)	Dose (ppm)			
	0	300	900	1800
Males				
13	1981.8 \pm 872.2	3059.5 \pm 1687.5	1995.6 \pm 699.5	1745.3 \pm 458.8
17	6123.6 \pm 3809.4	4089.1 \pm 2807.9	4091.8 \pm 2455.4	3451.2 \pm 1498.2
22	3058.2 \pm 784.2	3143.6 \pm 1013.8	3256.4 \pm 693.4	2567.1 \pm 748.0
60	7787.3 \pm 1256.0	8474.1 \pm 1384.8	8125.5 \pm 1759.7	8087.7 \pm 1745.8
Females				
13	1923.4 \pm 1214.7	1687.1 \pm 947.4	1847.7 \pm 657.6	1408.8 \pm 518.9
17	4967.2 \pm 3866.6	5486.6 \pm 3621.9	4283.8 \pm 2676.9	2840.0 \pm 2002.0
22	2709.9 \pm 722.5	3356.5 \pm 1598.1	2634.9 \pm 960.2	2954.8 \pm 1550.3
60	8893.5 \pm 1854.1	9820.4 \pm 2784.1	9246.4 \pm 1577.6	8559.8 \pm 1329.3

a Data were extracted from pages 74, 226-240 of the study report; n=10 pups.

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TABLE 8b. Mean (\pm SD) total session motor activity (# of rears) in F ₁ pups. ^a				
Interval (PND)	Dose (ppm)			
	0	300	900	1800
Males				
13	78.9 \pm 46.1	119.7 \pm 71.3	94.6 \pm 54.3	40.8 \pm 28.9* (\downarrow 48)
17	247.9 \pm 155.1	164.8 \pm 97.5	169.6 \pm 99.1	87.0 \pm 49.4** (\downarrow 65)
22	92.9 \pm 35.3	91.2 \pm 30.4	111.8 \pm 40.2	60.2 \pm 21.7* (\downarrow 35)
60	201.7 \pm 31.3	215.5 \pm 38.0	222.8 \pm 59.5	192.4 \pm 50.7
Females				
13	64.2 \pm 36.8	83.5 \pm 45.0	71.3 \pm 41.4	23.2 \pm 21.2** (\downarrow 64)
17	170.1 \pm 132.8	216.1 \pm 120.3	158.0 \pm 95.8	53.9 \pm 39.9** (\downarrow 68)
22	82.7 \pm 38.0	88.6 \pm 36.6	93.0 \pm 45.0	49.9 \pm 22.5 (\downarrow 40)
60	242.2 \pm 41.6	243.4 \pm 60.0	245.1 \pm 60.3	206.2 \pm 51.5

a Data were extracted from pages 74, 241-256 of the study report; n=10 pups. Numbers presented parenthetically are percent difference from controls (calculated by reviewers).

* Significantly different from controls at $p \leq 0.05$

** Significantly different from controls at $p \leq 0.01$

- c. **Auditory startle reflex habituation:** No treatment-related effects on total session peak amplitude, latency, or habituation were observed in Subset 3 animals of either sex at PND 24 or 60 (Table 9).

TABLE 9. Mean (\pm SD) overall (Blocks 1-5) acoustic startle max. amplitude (g) and latency to peak (msec) in F ₁ rats. ^a					
Dose (ppm)	Parameter	Males		Females	
		PND 24	PND 60	PND 24	PND 60
0	Max. Amp.	457.0 \pm 244.7	1396.6 \pm 862.2	439.6 \pm 212.0	980.9 \pm 302.9
	Latency	27.5 \pm 6.2	36.8 \pm 7.6	32.2 \pm 7.9	32.9 \pm 5.5
300	Max. Amp.	422.6 \pm 168.0	2145.8 \pm 2522.4	581.3 \pm 311.6	873.8 \pm 600.9
	Latency	28.8 \pm 6.9	43.3 \pm 15.1	33.5 \pm 9.8	33.0 \pm 6.5
900	Max. Amp.	554.6 \pm 334.0	2190.5 \pm 1507.9	416.5 \pm 156.2	947.1 \pm 486.6
	Latency	34.4 \pm 10.3	43.3 \pm 12.5	27.8 \pm 8.3	33.1 \pm 6.7
1800	Max. Amp.	348.0 \pm 181.5	2139.5 \pm 1558.2	320.1 \pm 152.3	909.7 \pm 369.0
	Latency	36.4 \pm 8.7	44.8 \pm 15.2	28.7 \pm 6.8	30.5 \pm 6.1

a Data were extracted from pages 77, 257-265 of the study report; n=10.

- d. **Learning and memory testing:** No treatment-related effects on learning, memory, and relearning and time to success were observed at any time point in either sex (Tables 10a and 10b). The decrease in the number of animals reaching criteria in the 900 ppm males during Trial 4 of the Learning 2 phase on PND 30 was unrelated to dose.

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TABLE 10a. Water maze performance (# of animals reaching criteria) in F ₁ rats. ^a									
Session/Parameter		Dose (ppm)							
		0	300	900	1800	0	300	900	1800
		Males				Females			
PND 23 and 30									
Learning 1	Trial 2	6	4	4	8	5	5	5	1
	Trial 3	7	5	5	7	7	7	6	5
	Trial 4	7	4	8	9	7	7	7	5
	Trial 5	7	5	8	10	8	7	9	6
	Trial 6	8	7	7	10	9	7	9	6
Memory		7	6	9	6	7	6	5	6
Learning 2	Trial 2	2	3	0	2	3	3	3	1
	Trial 3	3	2	1	2	4	4	6	3
	Trial 4	5	4	0*	3	5	3	5	8
	Trial 5	7	6	4	3	6	4	6	6
	Trial 6	5	6	4	5	5	5	5	7
PND 60 and 67									
Learning 1	Trial 2	7	4	5	4	5	7	7	6
	Trial 3	9	6	6	6	6	7	7	6
	Trial 4	8	8	7	7	6	6	7	7
	Trial 5	9	8	8	7	7	7	8	9
	Trial 6	10	8	7	9	6	8	9	8
Memory		8	9	5	8	7	8	8	7
Learning 2	Trial 2	1	1	2	1	0	3	0	1
	Trial 3	1	2	3	3	2	2	2	0
	Trial 4	2	3	3	2	4	4	2	1
	Trial 5	2	3	4	4	4	5	4	4
	Trial 6	2	4	6	3	5	4	4	3

^a Data were extracted from pages 79, 152, 153, 156, and 157 of the study report; n=10.

* Statistically significantly different from controls at $p \leq 0.05$

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TABLE 10b. Mean (±SD) water maze performance (Time [sec]) in F ₁ rats. ^a									
Session/Parameter		Dose (ppm)							
		0	300	900	1800	0	300	900	1800
		Males				Females			
PND 23 and 30									
Learning 1	Trial 2	25±20.2	27±21.9	39±56.9	20±11.5	47±43.2	28±22.9	31±20.6	48±34.0
	Trial 3	21±19.9	23±19.2	27±25.7	30±30.0	24±23.3	17±12.6	14±9.1	18±13.0
	Trial 4	18±18.1	16±5.2	14±11.1	15±10.9	15±8.5	15±16.1	18±14.0	20±26.9
	Trial 5	15±16.3	15±8.6	7±4.0	9±6.9	15±12.7	12±11.0	11±10.3	16±19.1
	Trial 6	10±7.8	14±7.3	8±6.5	8±3.7	14±19.0	7±4.6	8±6.3	16±6.8
Memory		8±6.1	14±7.7	7±3.0	11±9.2	12±10.8	16±11.9	12±7.6	11±4.5
Learning 2	Trial 2	19±9.7	26±18.0	21±9.7	21±15.6	14±9.2	16±12.5	16±7.0	34±44.2
	Trial 3	15±8.4	19±13.3	13±2.8	18±16.4	12±10.0	11±9.6	9±7.9	16±9.3
	Trial 4	10±5.8	12±7.0	13±3.6	16±13.1	10±6.0	11±6.1	10±7.8	10±12.5
	Trial 5	7±3.2	9±5.8	10±2.8	11±4.1	8±4.5	10±6.3	8±5.5	13±13.1
	Trial 6	11±10.0	7±4.2	11±6.6	9±3.2	11±6.9	9±7.8	7±4.8	8±4.8
PND 60 and 67									
Learning 1	Trial 2	25±28.4	20±9.7	22±17.8	17±8.8	19±16.7	16±8.7	22±24.3	16±8.3
	Trial 3	7±2.0	11±6.7	11±7.7	17±14.4	12±7.5	13±7.6	12±13.8	17±9.4
	Trial 4	8±4.1	10±8.1	7±2.7	10±8.5	10±9.8	16±19.0	10±9.3	13±6.3
	Trial 5	9±11.7	6±3.6	6±1.8	7±4.9	9±6.3	13±13.4	7±5.2	9±2.8
	Trial 6	5±1.9	14±22.5	7±5.6	7±6.2	9±5.0	16±26.4	6±5.0	16±12.7
Memory		7±5.7	15±30.3	12±11.8	10±9.3	9±5.0	9±6.5	8±5.1	12±7.6
Learning 2	Trial 2	31±30.9	36±61.8	17±8.3	24±16.0	23±10.0	13±4.4	16±10.8	19±8.3
	Trial 3	18±13.0	27±30.8	14±10.8	12±6.2	16±9.1	13±6.9	10±4.1	18±9.2
	Trial 4	14±9.2	36±39.5	15±16.8	14±7.7	15±8.6	14±10.5	12±5.5	14±5.9
	Trial 5	30±34.2	19±20.0	15±13.1	17±23.4	12±5.0	8±3.8	8±4.3	12±8.0
	Trial 6	19±22.8	29±34.9	10±7.4	16±16.6	12±7.1	9±4.6	10±7.6	18±22.9

a Data were extracted from pages 154, 155, 158, and 159 of the study report; n=10.

5. **Toxicokinetics:** Mean plasma concentrations of MCPA and 2-HMCPA (metabolites of the test material) in the dams and pups are presented in Tables 11a and 11b below.

The test material (MCPA 2-EHE) was not detectable in the plasma of the dams or pups. However, internal exposure was demonstrated by the considerable concentrations of MCPA and minor amounts of 2-HMCPA in the dams and their pups. The plasma concentrations of the metabolites were dose dependent and relatively constant during the last days of gestation and (except in the low dose) throughout lactation in the dams. The results provide clear evidence that the pups were exposed to the test material and/or its metabolites. The plasma concentrations of the metabolites were dose dependent and generally increased during the course of the lactation period. There were no marked differences between the male and female pups.

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TABLE 11a. Mean (\pm SD) plasma concentrations of MCPA and 2-HMCPA in dams. ^a			
Dose (ppm)	Time point	MCPA (μ g/mL plasma)	2-HMCPA (μ g/mL plasma)
300	GD 20	21.5 \pm 4.7	ND
200	LD 4	12.6 \pm 5.0	ND
	LD 11	19.3 \pm 7.3	ND
	LD 21	21.4 \pm 10.0	0.26
900	GD 20	57.3 \pm 15.5	ND
600	LD 4	42.9 \pm 8.0	ND
	LD 11	56.6 \pm 13.7	ND
	LD 21	47.9 \pm 12.3	0.24 \pm 0.10
1800	GD 20	131.9 \pm 34.3	0.68 \pm 0.50
1200	LD 4	99.1 \pm 35.4	ND
	LD 11	100.1 \pm 23.6	ND
	LD 21	112.6 \pm 24.7	0.21 \pm 0.40

a Data were extracted from pages 90, 827-830, 837-840, and 847 of the study report; n=10.

TABLE 11b. Mean (\pm SD) plasma concentrations of MCPA and 2-HMCPA in male and female pups. ^a					
Dose (ppm)	Time point	Mean plasma concentration (μ g/mL plasma)			
		Males		Females	
		MCPA	2-HMCPA	MCPA	2-HMCPA
200	PND 4	4.07 \pm 1.3	ND	2.70 \pm 1.7	ND
	PND 11	8.17 \pm 4.4	ND	9.56 \pm 6.1	ND
	PND 21	32.5 \pm 12.6	ND	23.75 \pm 6.2	ND
600	PND 4	24.65 \pm 9.1	ND	25.16 \pm 12.9	ND
	PND 11	40.93 \pm 11.3	ND	39.26 \pm 11.5	ND
	PND 21	89.76 \pm 18.8	0.27 \pm 0.10	99.69 \pm 26.3	0.21 \pm 0.10
1200	PND 4	120.1 \pm 34.0	1.70 \pm 1.5	110.9 \pm 46.7	1.5 \pm 1.3
	PND 11	106.2 \pm 21.4	0.93 \pm 0.40	104.9 \pm 27.4	0.85 \pm 0.40
	PND 21	164.3 \pm 26.4	0.55 \pm 0.40	173.2 \pm 22.1	0.70 \pm 0.40

a Data were extracted from pages 91, 831-836, 841-846, and 848 of the study report; n=10.

6. Postmortem results

- a. **Brain weights:** At 1800 ppm, there were statistically significant decreases in absolute brain weights in both sexes on PND 22 (Table 12). Although brain weight decreases do not scale with body weight decreases in adults (i.e. brain weight preserve), the brain weight decreases seen in these pups at this dose could not be attributed solely to treatment, since significant decreases in pup body weights were seen during their developing period (i.e. PNDs 1-21; see Tables 5a and 5b). The brain weight decreases did not worsen with age (i.e. same magnitude of decreases for both PND 22 and 60).

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TABLE 12. Mean (\pm SD) brain weights in F ₁ rats ^a				
Parameter	Dose (ppm)			
	0	300	900	1800
Males				
PND 22				
Terminal body weight (g)	51.47 \pm 2.2	48.81 \pm 3.1* (\downarrow 5)	49.85 \pm 5.0	37.85 \pm 5.9** (\downarrow 26)
Brain weight (g)	1.68 \pm 0.08	1.68 \pm 0.08	1.64 \pm 0.06	1.56 \pm 0.06** (\downarrow 7)
Brain-to-body weight ratio (%)	3.27 \pm 0.19	3.45 \pm 0.21	3.32 \pm 0.35	4.21 \pm 0.63** (\uparrow 29)
PND 60 (\pm 2)				
Terminal body weight (g)	284.5 \pm 27.8	291.2 \pm 24.9	283.2 \pm 17.6	251.6 \pm 18.8** (\downarrow 12)
Brain weight (g)	2.02 \pm 0.09	1.97 \pm 0.05	2.00 \pm 0.10	1.88 \pm 0.09** (\downarrow 7)
Brain-to-body weight ratio (%)	0.72 \pm 0.08	0.68 \pm 0.05	0.71 \pm 0.04	0.75 \pm 0.04
Females				
PND 22				
Terminal body weight (g)	49.04 \pm 4.6	49.41 \pm 4.4	49.46 \pm 5.1	34.92 \pm 3.5** (\downarrow 29)
Brain weight (g)	1.57 \pm 0.07	1.60 \pm 0.08	1.56 \pm 0.08	1.46 \pm 0.06** (\downarrow 7)
Brain-to-body weight ratio (%)	3.23 \pm 0.27	3.25 \pm 0.23	3.17 \pm 0.24	4.24 \pm 0.54** (\uparrow 31)
PND 60 (\pm 2)				
Terminal body weight (g)	179.5 \pm 19.8	180.1 \pm 11.5	174.1 \pm 14.5	172.9 \pm 11.8
Brain weight (g)	1.86 \pm 0.12	1.92 \pm 0.08	1.87 \pm 0.06	1.81 \pm 0.08
Brain-to-body weight ratio (%)	1.04 \pm 0.09	1.07 \pm 0.06	1.08 \pm 0.09	1.05 \pm 0.06

a Data were extracted from pages 265-272 of the study report; n=10. Numbers presented parenthetically are percent difference from controls (calculated by reviewers).

* Significantly different from controls at $p \leq 0.05$

** Significantly different from controls at $p \leq 0.01$

b) Neuropathology

- 1. Macroscopic examination:** No gross lesions or treatment-related differences in brain lengths or widths were observed on PND 22 or at termination in either sex. The decreases ($p \leq 0.01$) noted in brain length in the males on PND 22 and 60 (± 2) and brain width in the females on PND 22 in the 1800 ppm group were minor ($\downarrow 3$ -4%) and considered to be secondary to the decreased terminal body weights in these animals rather than a direct effect of treatment on brain development.
- 2. Microscopic examination:** No treatment-related microscopic lesions were noted at any dose in either sex on PND 22 or at termination.

No treatment-related differences in morphometric brain measurements were noted in the 1800 ppm animals compared to controls on PND 22 or 62 (Tables 13a and 13b). The only statistically significant difference from controls was a decrease in the thickness of the folium pyramis ($\downarrow 8$, $p \leq 0.05$) in the 1800 ppm females on PND 62.

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TABLE 13a. Mean (\pmSD) morphometric brain measurements in male F₁ rats ^a				
Parameter	Dose (ppm)			
	0	300	900	1800
PND 22				
Frontal Cortex, left (μ m)	1470 \pm 132	NM	NM	1579 \pm 85
Frontal Cortex, right (μ m)	1485 \pm 105	NM	NM	1623 \pm 113
Parietal Cortex, left (μ m)	1477 \pm 191	NM	NM	1533 \pm 84
Parietal Cortex, right (μ m)	1419 \pm 127	NM	NM	1524 \pm 105
Caudate nucleus, left (μ m)	3015 \pm 261	NM	NM	3093 \pm 260
Caudate nucleus, right (μ m)	3016 \pm 170	NM	NM	3180 \pm 123
Corpus callosum (μ m)	204 \pm 24	NM	NM	208 \pm 24
Hippocampus, left (μ m)	1331 \pm 205	NM	NM	1427 \pm 74
Hippocampus, right (μ m)	1312 \pm 198	NM	NM	1439 \pm 65
Cerebellum (folium pyramis, μ m)	327 \pm 36	NM	NM	343 \pm 37
PND 62 (Termination)				
Frontal Cortex, left (μ m)	1821 \pm 87	NM	NM	1826 \pm 98
Frontal Cortex, right (μ m)	1814 \pm 82	NM	NM	1812 \pm 49
Parietal Cortex, left (μ m)	1812 \pm 131	NM	NM	1827 \pm 80
Parietal Cortex, right (μ m)	1841 \pm 113	NM	NM	1771 \pm 65
Caudate nucleus, left (μ m)	3912 \pm 191	NM	NM	4005 \pm 111
Caudate nucleus, right (μ m)	3804 \pm 219	NM	NM	4003 \pm 114
Corpus callosum (μ m)	274 \pm 29	NM	NM	279 \pm 29
Hippocampus, left (μ m)	1824 \pm 121	NM	NM	1860 \pm 79
Hippocampus, right (μ m)	1856 \pm 131	NM	NM	1866 \pm 57
Cerebellum (folium pyramis, μ m)	403 \pm 44	NM	NM	391 \pm 36

a Data were extracted from pages 282 and 284; n=10.

NM Not measured

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TABLE 13b. Mean (±SD) morphometric brain measurements in female F₁ rats ^a				
Parameter	Dose (ppm)			
	0	300	900	1800
PND 22				
Frontal Cortex, left (µm)	1475±107	NM	NM	1578±56
Frontal Cortex, right (µm)	1484±102	NM	NM	1542±112
Parietal Cortex, left (µm)	1447±131	NM	NM	1538±117
Parietal Cortex, right (µm)	1446±131	NM	NM	1470±80
Caudate nucleus, left (µm)	3028±294	NM	NM	3150±228
Caudate nucleus, right (µm)	2968±221	NM	NM	3165±124
Corpus callosum (µm)	208±36	NM	NM	214±30
Hippocampus, left (µm)	1398±84	NM	NM	1432±182
Hippocampus, right (µm)	1366±83	NM	NM	1439±138
Cerebellum (folium pyramis, µm)	327±19	NM	NM	351±32
PND 62 (Termination)				
Frontal Cortex, left (µm)	1684±103	NM	NM	1718±65
Frontal Cortex, right (µm)	1700±79	NM	NM	1759±76
Parietal Cortex, left (µm)	1650±80	NM	NM	1719±59
Parietal Cortex, right (µm)	1609±92	NM	NM	1685±81
Caudate nucleus, left (µm)	3746±232	NM	NM	3957±137
Caudate nucleus, right (µm)	3844±176	NM	NM	3790±176
Corpus callosum (µm)	255±39	NM	NM	231±31
Hippocampus, left (µm)	1714±131	NM	NM	1779±100
Hippocampus, right (µm)	1686±139	NM	NM	1804±113
Cerebellum (folium pyramis, µm)	415±35	NM	NM	381±34* (↓8)

^a Data were extracted from pages 283 and 285; n=10.

NM Not measured

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS:

The investigators concluded that no evidence of developmental neurotoxicity was observed at any dose in either sex. The NOAEL for developmental neurotoxicity is 1800 ppm and 300 ppm for systemic toxicity in dams and pups due to decreased body weight gain.

B. REVIEWER COMMENTS:

The reviewers disagreed with the Sponsor concerning setting the LOAEL at 900 ppm for maternal and offspring toxicity. At 900 ppm, body weight gains were decreased ($p \leq 0.01$) by 11% during GD 6-13, contributing to a decrease ($p \leq 0.05$) of 8% for GD 6-20 without corresponding significant decreases in body weight. Body weight gains were decreased ($p \leq 0.05$) by 7% during PND 14-21, contributing to a decrease ($p \leq 0.05$) of 6% for PND 4-21 without corresponding significant decreases in body weight. In the absence of additional evidence of toxicity at this dose, this minor effect on body weight gain in the dams and offspring was considered treatment-related but not adverse.

No evidence of developmental neurotoxicity was observed at any dose.

The maternal LOAEL is 1800 ppm (equivalent to 156 mg/kg/day); based on decreases in body weight, body weight gain, and food consumption. The maternal NOAEL is 900 ppm (equivalent to 83 mg/kg/day).

The offspring LOAEL is 1800 ppm (equivalent to 156 mg/kg/day), based on decreases in body weight and body weight gain and an increase in pup mortality. The offspring NOAEL is 900 ppm (equivalent to 83 mg/kg/day).

This study is classified **Acceptable/Non-Guideline** and may be used for regulatory purposes, however, it does not satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300; OECD 426) at this time pending a comprehensive review of all available positive control data.

C. STUDY DEFICIENCIES: No major deficiencies were noted.